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Ezetimibe alone or in combination with simvastatin increases small dense low-density lipoproteins in healthy men: a randomized trial

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Abstract: AIMS: The predominance of small dense low-density lipoproteins (sdLDLs) has been associated with increased cardiovascular risk. The effect of ezetimibe on LDL subfraction distribution has not been fully elucidated. This study assessed by gradient gel electrophoresis the effects of ezetimibe alone, simvastatin alone, and their combination on sdLDL subfraction distribution. **METHODS AND RESULTS:** A single-centre, randomized, parallel three-group open-label study was performed in 72 healthy men with a baseline LDL-cholesterol (LDL-C) concentration of 111 ± 30 mg/dL (2.9 ± 0.8 mmol/L). They were treated with ezetimibe (10 mg/day, $n = 24$), simvastatin (40 mg/day, $n = 24$), or their combination ($n = 24$) for 14 days. Blood was drawn before and after the treatment period. Generalized estimating equations were used to assess the influence of drug therapy on LDL subfraction distribution, controlling for within-subject patterns (clustering). We adjusted for age, body mass index, and baseline concentrations of LDL-C and triglycerides. Ezetimibe alone changed LDL subfraction distribution towards a more atherogenic profile by significantly increasing sdLDL subfractions (LDL-IVA $+14.2\%$, $P = 0.0216$ and LDL-IVB $+16.7\%$, $P = 0.039$; fully adjusted Wald χ^2 test). In contrast, simvastatin alone significantly decreased the LDL-IVB subfraction (-16.7% , $P = 0.002$). This effect was offset when simvastatin was combined with ezetimibe (LDL-IVB $+14.3\%$, $P = 0.44$). All three treatments decreased the large, more buoyant LDL-I subfraction, the effects of ezetimibe being the most pronounced (ezetimibe -13.9% , $P < 0.0001$; combination therapy -7.3% , $P = 0.0743$; simvastatin -4.6% , $P < 0.0001$). **CONCLUSION:** In healthy men, treatment with ezetimibe alone is associated with the development of a pro-atherogenic LDL subfraction profile. Potentially atheroprotective effects of simvastatin are offset by ezetimibe. This study is registered with ClinicalTrials.gov, identifier no. NCT00317993.

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1 **Ezetimibe Alone or in Combination with Simvastatin Increases Small Dense Low-Density**
2 **Lipoproteins in Healthy Men – A Randomized Trial**

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ABSTRACT

Context The predominance of small dense low-density lipoproteins (LDL) has been associated with increased cardiovascular risk. The effect of ezetimibe on LDL subfraction distribution has not been fully elucidated.

Objective To determine the effects of ezetimibe alone, simvastatin alone, and their combination on small dense LDL subfraction distribution, assessed by gradient gel electrophoresis.

Design, Setting, and Participants Monocenter randomized parallel 3-group open-label study in 72 healthy men with a baseline LDL cholesterol concentration of 111 ± 30 mg/dL (2.9 ± 0.8 mmol/L).

Interventions 14-day treatment with ezetimibe (10 mg/day, $n = 24$), simvastatin (40 mg/day, $n = 24$) or their combination ($n = 24$). Blood was drawn before and after the treatment period.

Main Outcome Measures Generalized estimating equations were used to assess the influence of drug therapy on LDL subfraction distribution, controlling for within-subject patterns (clustering). The analyses were adjusted for age, body mass index, and baseline concentrations of LDL cholesterol and triglycerides.

Results Ezetimibe alone changed LDL subfraction distribution towards a more pro-atherogenic profile by significantly increasing small dense LDL subfractions (LDL-IVA +14.2%, $P = .0216$ and LDL-IVB +16.7%, $P = .039$; fully adjusted Wald chi-square test). In contrast, simvastatin alone significantly decreased the LDL-IVB subfraction (−16.7%, $P = .002$). This effect was cancelled when simvastatin was combined with ezetimibe (LDL-IVB +14.3%, $P = .44$). All 3 treatments decreased the large, more buoyant LDL-I subfraction, the effects of ezetimibe being the most pronounced (ezetimibe −13.9%, $P < .0001$; combination therapy −7.3%, $P = .0743$; simvastatin −4.6%, $P < .0001$). None of the treatments influenced LDL particle size.

Conclusions In healthy men, treatment with ezetimibe alone is associated with the development of a pro-atherogenic LDL subfraction profile. The potentially atheroprotective effects of simvastatin are cancelled by ezetimibe.

Trial Registration. ClinicalTrials.gov Identifier: NCT00317993

INTRODUCTION

Ezetimibe represents the first of a new class of lipid-lowering agents, the cholesterol absorption inhibitors. It is able to reduce low-density lipoprotein cholesterol (LDL-C) by 15–25% when given as monotherapy or added on an ongoing statin treatment.^{1,2} Due to the complementary mechanism of action of ezetimibe and statins (inhibition of cholesterol absorption and synthesis, respectively) and to their additive effects on LDL-C-lowering, their combination is widely used to achieve reductions in LDL-C of up to 60%.^{3,4}

However, a substantial body of evidence suggests that the “quality” and not only the “quantity” of LDL exert a direct influence on cardiovascular risk (reviewed in ^{5,6}). LDL consists of a set of discrete subfractions with distinct molecular properties, among them size and density. In normal subjects, seven major LDL subfractions can be identified [I (large), IIA and IIB (medium), IIIA and IIIB (small), IVA and IVB (very small)]. LDL-I is the largest and least dense and LDL-IVB is the smallest most dense particle.⁷ The predominance of small dense LDL (sdLDL) and of small LDL particle size (diameter <258 Å) have been associated with increased cardiovascular risk.^{5,8-11} In this context, the LDL-IVB subfraction has been found to be the single best lipoprotein predictor for atherosclerotic disease progression.¹²

Statins have been shown to have either no or only a moderate effect on LDL subclass distribution or particle size.¹³ Few studies have so far assessed the effects of ezetimibe on LDL particle size and/or subfraction distribution (as reviewed in ¹⁴), with conflicting results. Furthermore, most of these trials included subjects with concomitant metabolic disorders such as obesity, hypercholesterolemia, diabetes, and the metabolic syndrome, and with a variety of co-medications with unknown effects on lipoproteins.

The recent ENHANCE trial found that, although the addition of ezetimibe (10 mg/day) to simvastatin (80 mg/day) in patients with heterozygous familial hypercholesterolemia caused an additional 16.5% reduction in LDL-C, it did not significantly affect the primary end point, i.e. the mean change in intima-media thickness (IMT), compared to simvastatin monotherapy.¹⁵ Subsequently, the value of ezetimibe in the arena of cardiovascular prevention was questioned and although various theories have been proposed to explain these surprising results, the actual reasons remain unclear.^{16,17}

Purpose of the present study was to test the hypothesis that ezetimibe may alter LDL subfraction distribution and particle size to a pro-atherogenic profile in healthy subjects.

METHODS

Study Design

LDL particle size and subfractions were analyzed from frozen samples of a monocenter, randomized, parallel 3-group open-label study that investigated the effects of ezetimibe and simvastatin, alone or in combination, on lipid metabolism.^{4,18} A total of 72 subjects were randomized to receive ezetimibe (10 mg/day), simvastatin (40 mg/day) or ezetimibe (10 mg/day) plus simvastatin (40 mg/day) for 2 weeks (n = 24 for each group). Ezetimibe and simvastatin were taken once a day in the evening. Blood was drawn before the initiation of treatment and at the end of the treatment period and the samples were analyzed in a blinded manner.

Subjects

Inclusion criteria were age between 18 and 60 years, body mass index (BMI) between 18.5 and 30 kg/m², fasting LDL-C concentrations <190 mg/dL, triglyceride concentrations <250 mg/dL and normal blood pressure (<140/90 mmHg). Subjects who had received lipid-lowering drugs within 12 weeks prior to study entry, those with a history of excessive alcohol intake, liver disease, renal dysfunction (glomerular filtration rate <60 mL/min), coronary heart disease, diabetes mellitus or other endocrine disorders, eating disorders, history of recent substantial (>10%) weight change, history of obesity (BMI >35 kg/m²) or taking medications known to affect lipoprotein metabolism were excluded from the study.

The protocol was approved by the Ethics Committee of the University of Cologne, and all subjects gave written informed consent. The study conformed to the Declaration of Helsinki. All subjects completed the study. Body weight did not change in any treatment group. The subjects did not use any extra medications, had no illnesses and did not deviate from the study protocol. No serious side effects were reported.

Biochemical Analyses

Blood was drawn by venipuncture in the morning after a 12 h fast to obtain serum for analysis of lipids. Total cholesterol, LDL-C and HDL-C as well as triglycerides were determined by enzymatic methods (CHOD-PAP and GPO-PAP; Roche Diagnostics, Mannheim, Germany) on the day of blood collection in the laboratories of the Cologne University Medical Center (inter-assay coefficient of variation for total cholesterol, LDL-C, HDL-C, and triglycerides were 1.09, 2.79, 0.81, and 1.72%, respectively). Serum was obtained by centrifugation at 3000 rpm for 30 min at 4°C within 15 min after venipuncture and aliquots were stored immediately after at –80°C for future analysis.

Nondenaturing polyacrylamide gradient gel electrophoresis (GGE) of serum was performed in the laboratory of K.B. at the University Hospital Zurich, Switzerland, in a blinded manner. Samples were shipped from Germany in dry ice and immediately analyzed by GGE without re-freezing. GGE was performed at 10–14°C in 2–16% polyacrylamide gradient gels. Gels were subjected to electrophoresis for 24 h at 125 V in tris borate buffer (pH 8.3) as previously described.^{7;19} Gels were fixed and stained for lipids in a solution containing oil red O in 60% ethanol at 55°C. Gels were placed on a light source and photographed using a Luminescent Image Analyzer, LAS-3000 of Fujifilm. Migration distance for each absorbance peak was determined and the molecular diameter corresponding to each peak was calculated from a calibration curve generated from the migration distance of size standards of known diameter, which includes carboxylated latex beads (Duke Scientific, Palo Alto, CA), thyroglobulin and apoferritin (HMW Std, Pharmacia, Piscataway, NJ) having molecular diameters of 380 Å, 170 Å and 122 Å, respectively, and lipoprotein calibrators of previously determined particle size. LDL subfraction distribution (LDL-I, -IIA, -IIB, -IIIA, -IIIB, -IVA and -IVB) as percentage of total LDL was calculated as previously described.⁷

Statistical Analysis

Descriptive data are presented as mean (SD) unless otherwise stated. We performed multivariate analyses using generalized estimating equations to assess the influence of therapy on LDL subclass distribution, controlling for within-subject patterns (clustering). We adjusted for age, body mass index and baseline concentrations of LDL-C and triglycerides. Statistical analyses were conducted using

Stata version 9 (StataCorp, College Station, TX). We used Stata's xtgee command to model panel data. All reported *P* values were calculated two-sided.

RESULTS

Baseline subject characteristics are shown in Table 1 and were not different among the 3 treatment groups. The flow of participants through the trial is shown in Figure 1. All subjects completed the study and the adherence was excellent, as based on pill counts (mean [SD] adherence, 99.1 [3.7]%). As shown in Table 2, total cholesterol and LDL-C levels significantly decreased in all treatment groups ($P < .001$ for all), while triglycerides decreased only in the groups receiving simvastatin. HDL-C concentrations remained unchanged in all groups.

Significant changes in LDL subfraction distribution were observed in all treatment groups after adjusting for age, body mass index and baseline concentrations of LDL-C and triglycerides (Wald chi square $P < .05$). The results are depicted in Figure 2. Adjusted comparisons within individual subclasses showed that ezetimibe treatment significantly increased smaller more dense LDL-IIB (+11.2%), LDL-IIIA (+19.5%), LDL-IIIB (+11.9%), LDL-IVA (+14.2%), and LDL-IVB (+16.7%) (Table 2, Figure 2A). Combination treatment (ezetimibe plus simvastatin) significantly increased LDL-IIIB (+27%) and in LDL-IVA (+28.5%) (Table 2, Figure 2B). Treatment with simvastatin alone significantly increased LDL-IIB (+11.3%), LDL-IIIA (+15.4%), LDL-IIIB (+15.4%), and LDL-IVA (+2.5%), but significantly decreased LDL-IVB, the most atherogenic LDL subfraction (−16.7%, $P = .002$) (Figure 2C). Interestingly, this effect was cancelled when ezetimibe was added to simvastatin (Table 2, Figure 2B and 3).

All treatments decreased the larger, more buoyant LDL-I (Table 2, Figures 2A, B and C). The decrease was most pronounced in the ezetimibe alone group (−13.9%), followed by the combination group (−7.3%) and smallest in the simvastatin alone group (−4.6%; Figure 3).

In multivariate analyses there was a significant influence of baseline LDL-C concentrations on changes in LDL subfraction distribution in the ezetimibe alone group. The increase in atherogenic small dense LDL was more pronounced when baseline LDL-C was higher and vice versa (data not shown).

None of the treatments had an effect on LDL particle size (Table 2).

COMMENT

One essential finding of our study is that treatment with ezetimibe alone or in combination with a statin, increases sdLDL proportions, thus leading to the development of a pro-atherogenic LDL subfraction profile. Small dense LDL has been accepted as an emerging cardiovascular risk factor by the National Cholesterol Education Program Adult Treatment Panel III.²⁰ Moreover, a consensus statement endorsed by the American Diabetes Association and the American College of Cardiology advocated measuring LDL particle concentration in subjects at high risk for cardiometabolic disorders and pointed out the pro-atherosclerotic effects of sdLDL.²¹ The mechanisms through which sdLDL may promote atherosclerosis include increased endothelial permeability, impaired clearance from the circulation, easier oxidation and glycation and increased ability to bind to proteoglycans in the vessel wall.^{22;23}

Although it cannot be fully excluded that the increased risk associated with smaller LDL phenotype may also be a consequence of the broader pathophysiology of which sdLDL are a part (e.g. high triglycerides, low HDL-C, increased LDL particle number, obesity, insulin resistance, diabetes, metabolic syndrome),^{22;24-26} some studies have shown that sdLDL are a strong and independent predictor of coronary artery disease (CAD).²⁷ Other studies have investigated whether the therapeutic modification of LDL subfractions may be significantly associated with reduced cardiovascular risk. Such investigations used angiographic changes as outcome variables and have reported benefit in patients with a predominance of sdLDL who received treatment such as statins and bile acid-binding resins that tend to reduce the amount of such particles.^{8;28;29}

In fact, various lipid-lowering drugs are able to favourably alter sdLDL, and fibrates and nicotinic acid seem to be the most effective in this respect (reviewed in ^{30;31}). As we were also able to show in the present study, simvastatin has been found to have either no or only a marginal net effect on LDL subfraction distribution.¹³ This is true for the majority of the statins.³² Interestingly, rosuvastatin, the latest statin to be introduced in the market, seems to be more efficient in modulating plasma lipids and LDL subfractions (reviewed in ^{13;33}). By contrast, the effects of the cholesterol

absorption inhibitor ezetimibe on LDL size and subfraction distribution have been contradictory. Ezetimibe monotherapy was found to be associated with a small but significant decrease in sdLDL concentrations and increase in LDL particle size in patients with primary dyslipidemia,³⁴ mixed hyperlipidemia,³⁵ and in obese and overweight patients with hypercholesterolemia.³⁶ On the other hand, Ose et al. found no effects of ezetimibe monotherapy on sdLDL concentrations and LDL particle size in patients with hypercholesterolemia.³⁷ Moreover, in patients with mixed hyperlipidemia Tribble et al. found that ezetimibe caused reductions in both, the large and sdLDL subfractions, and had no effects on LDL particle size.³⁸ Furthermore, Geiss et al. found no effect of ezetimibe on LDL subfraction distribution in patients concomitantly treated with LDL apheresis and statins.³⁹ To our knowledge this is the first study to examine whether ezetimibe modulates LDL size and subfraction distribution in healthy individuals, a model which in a sense reflects ezetimibe's "true" effects on a healthy metabolic background.

In the present study we investigated a group of healthy men to assess the effects of ezetimibe, simvastatin and their combination on LDL particle size and subfraction distribution. We were able to show that treatment with ezetimibe alone or in combination with a statin did not alter LDL particle size but altered the LDL subfraction distribution towards increased concentrations of atherogenic small dense particles. Although simvastatin alone also increased LDL-III subfractions, it significantly decreased the smallest, most dense LDL fraction (LDL-IVB), which has been found to be the best lipoprotein predictor of atherosclerotic disease progression, even if it represents only a minor fraction of total LDL.¹² This potentially atheroprotective effect of simvastatin was cancelled when ezetimibe was coadministered. These findings may, at least partially, explain the lack of additional benefit of ezetimibe added to simvastatin on atherosclerosis progression, measured as changes in intima-media thickness (IMT), despite a significant additional reduction in LDL-C levels, observed in the ENHANCE study.¹⁵ Although there is still no consensus on the clinical significance of surrogate markers of cardiovascular risk, such as IMT,⁴⁰ it should be pointed out that data from a subsequent study with ezetimibe were also disappointing; the Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) study showed that treatment with ezetimibe plus simvastatin significantly reduced LDL-C

concentrations in patients with aortic stenosis, compared to placebo, but did not affect the composite primary end point of aortic-valve events and ischemic events.⁴¹

Interestingly, we found that under treatment with ezetimibe alone, a significant association existed between baseline LDL-C concentrations and the pro-atherogenic changes of the LDL subfractions. Considering that the population of the current study was normocholesterolemic and that ezetimibe is prescribed to patients with much higher LDL-C levels, it can be postulated that the pro-atherogenic effects of ezetimibe would be even more pronounced in the latter population. Although it could be argued that ezetimibe is usually prescribed along with a statin, a group in which such an association was not observed, it should be pointed out that ezetimibe monotherapy is a widely used alternative for the treatment of hypercholesterolemia in patients with statin intolerance.⁴²

Limitation of the study is the fact that the clinical relevance of our findings remains to be established. Strengths of the study include its randomized design and robust statistical methodology, the blinded measurements of LDL subclasses, and the use of a “drug-naïve” population, devoid of co-medications and co-morbidities, which could potentially alter lipid metabolism, and excellent treatment adherence. Treatment duration was relatively short, which does not exclude that the observed effects could be even more pronounced during long-term treatment.

In conclusion, our findings suggest that treatment with ezetimibe, alone or in combination with simvastatin, is associated with the development of a pro-atherogenic LDL subfraction profile. Moreover, ezetimibe when given in combination with simvastatin cancels potentially anti-atherogenic effects of simvastatin. Cardiovascular event outcome trials, which are underway, will hopefully provide additional insights into the effects of ezetimibe on cardiovascular events.

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Author Contributions: Drs Berthold and Gouni-Berthold had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Rizzo, Berthold, Gouni-Berthold.

Acquisition of data: Berneis, Krone, Gouni-Berthold.

Analysis and interpretation of data: Berneis, Rizzo, Berthold, Spinas, Gouni-Berthold.

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Statistical analysis: Rizzo, Berthold.

Administrative, technical, or material support: Spinas, Krone, Gouni-Berthold.

Study supervision: Spinas, Gouni-Berthold.

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FIGURE LEGENDS

Figure 1. Flow of Participants Through the Trial

Figure 2. LDL Subclass Distribution (in Percent) and Changes from Baseline

LDL subclass distribution in the ezetimibe monotherapy group (A), combination treatment group (ezetimibe plus simvastatin) (B), and simvastatin monotherapy group (C). Significant changes, as determined by generalized estimating equations (Wald chi square P values), adjusting for age, body mass index, baseline LDL cholesterol and triglycerides, are indicated by asterisks ($*P < .05$, $(*)P < .1$). Data shown are mean values (SEM).

Figure 3. Percent Change from Baseline in LDL Subfractions in the 3 Treatment Groups.

Data shown are mean values (SEM).

TABLES

Table 1. Demographic Data and Biochemical Baseline Characteristics of the Study Participants

Age, y	32 (9)
Height, cm	181 (7)
Weight, kg	85 (12)
BMI, kg/m ²	25.7 (3.2)
Fasting plasma glucose, mg/dL	88 (8)
Smoking status	
Current smoker, n (%)	21 (29)
Ex-smoker, n (%)	9 (12.5)
Never smoker, n (%)	42 (58.3)
Serum lipoproteins	
Total cholesterol, mg/dL	189 (35)
LDL cholesterol, mg/dL	111 (30)
HDL cholesterol, mg/dL	64 (15)
Triglycerides, mg/dL	95 (43)
LDL particle size, Å	276 (9)
LDL subclasses	
LDL-I, %	37.2 (6.7)
LDL-IIA, %	17.4 (3.6)
LDL-IIB, %	16.0 (4.3)
LDL-IIIA, %	8.6 (20.5)
LDL-IIIB, %	4.7 (1.0)
LDL-IVA, %	6.8 (1.6)
LDL-IVB, %	7.3 (2.1)

Abbreviations: BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein.

Data are presented as mean (SD) or counts (percentages). There were no significant differences between the 3 treatment groups.

Table 2. Plasma Lipids, LDL Size, and LDL Subfraction Distribution Before and After Treatment

Parameter	Ezetimibe Only				Ezetimibe plus Simvastatin				Simvastatin Only			
	Before Therapy	After Therapy	Mean Percent Change	<i>P</i> Value*	Before Therapy	After Therapy	Mean Percent Change	<i>P</i> Value*	Before Therapy	After Therapy	Mean Percent Change	<i>P</i> Value*
Lipoprotein concentrations												
Total cholesterol, mg/dL	180 (28)	159 (23)	−11.2 (9.7)	.0002	194 (41)	121 (25)	−36.9 (8.1)	<.0001	194 (34)	145 (24)	−24.7 (7.9)	<.0001
LDL cholesterol, mg/dL	105 (23)	80 (16)	−22.1 (10.2)	<.0001	116 (35)	47 (19)	−59.6 (9.7)	<.0001	113 (30)	67 (22)	−40.7 (11.5)	<.0001
HDL cholesterol, mg/dL	64 (13)	65 (16)	+1.7 (11)	.35	61 (14)	60 (14)	−1.5 (8.5)	.23	65 (18)	65 (16)	+7 (11.1)	.88
Triglycerides, mg/dL	78 (32)	88 (49)	+27 (79)	.57	106 (48)	90 (36)	−8.9 (29.7)	.0288	101 (45)	82 (39)	−11.8 (39.9)	.0386
LDL particle size, Å	279 (7)	279 (10)	+2 (3.3)	.73	273 (9)	276 (8)	+1.1 (2.6)	.0975	277 (11)	276 (8)	−.4 (1.9)	.22
LDL subclass composition												
LDL-I, %	39.6 (5.5)	33.9 (5.9)	−13.9 (11.8)	<.0001	35.4 (7.3)	31.8 (4.7)	−7.3 (19.1)	.0743	36.8 (6.7)	34.5 (5.7)	−4.6 (16.7)	<.0001
LDL-IIA, %	16.3 (2.9)	16.1 (2.2)	−.5 (9.8)	.89	19.1 (4.6)	18.7 (4.3)	+6 (20.1)	.73	16.8 (2.3)	17.3 (2.0)	+4.0 (10.1)	.59
LDL-IIB, %	14.2 (1.8)	15.8 (3.1)	+11.2 (15.8)	.0003	18.1 (5.3)	17.6 (3.2)	+3.4 (27.2)	.37	15.7 (4.1)	17.1 (3.1)	+11.3 (16.3)	.0002
LDL-IIIA, %	10.3 (1.6)	12.1 (1.8)	+19.5 (21.0)	<.0001	10.6 (2.4)	11.5 (1.8)	+13.6 (28.5)	.0609	11.3 (3.5)	12.5 (2.6)	+15.4 (24.3)	<.0001
LDL-IIIB, %	5.1 (1.0)	5.6 (1.0)	+11.9 (19.2)	.0021	4.3 (1.0)	5.2 (1.2)	+27.0 (30.0)	.0017	4.9 (.9)	5.6 (1.0)	+17.3 (24.1)	.0011
LDL-IVA, %	7.2 (1.4)	8.2 (2.0)	+14.2 (25.9)	.0216	6.2 (1.8)	7.8 (2.2)	+28.5 (32.1)	.0002	7.0 (1.6)	6.9 (1.6)	+2.5 (30.7)	.0179
LDL-IVB, %	7.4 (1.7)	8.3 (2.2)	+16.7 (40.4)	.0392	6.5 (2.2)	7.3 (2.9)	+14.3 (30.8)	.44	8.0 (2.2)	6.1 (1.8)	−16.7 (37.4)	.002

Data are presented as mean (SD). Each group comprised n = 24 subjects. *Wald chi square test after adjusting for age, body mass index, baseline LDL cholesterol and triglycerides.

Figure 1

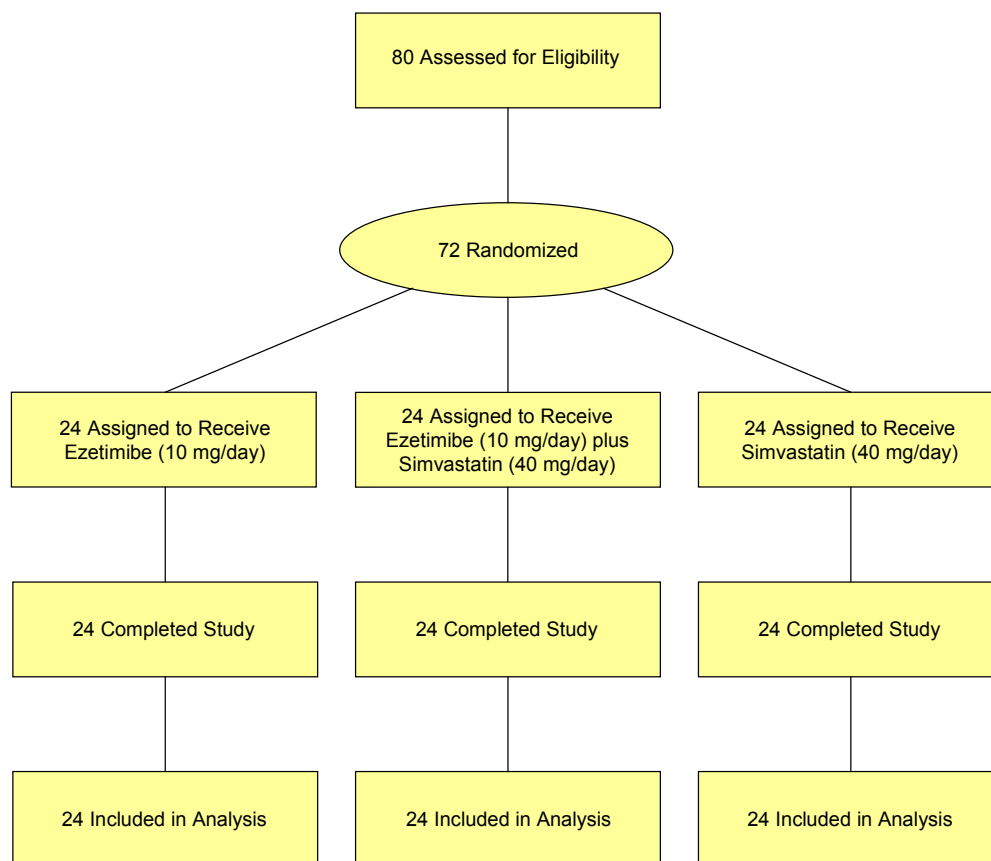


Figure 2

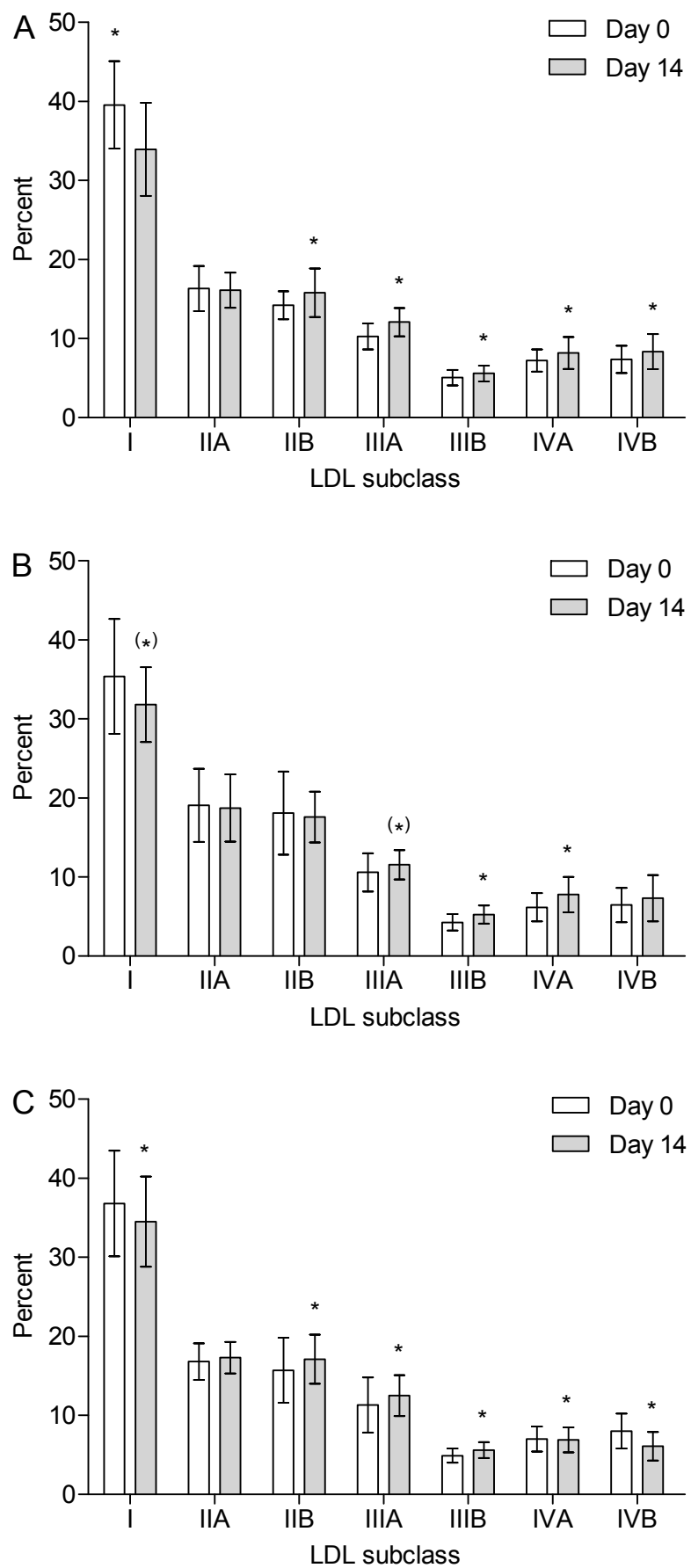


Figure 3

